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An On-Site Immunoassay for Detecting PCB in Soil

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Although the production and use of polychlorinated biphenyls (PCB) was banned in the United States over ten years ago, identification and remediation of PCB-contaminated sites continue to be a problem. Conventional laboratory analysis of soil samples is time consuming and costly due to the need for sophisticated equipment, highly trained technicians and transfer of samples from site to laboratory. Immunoassay technology (Van Emon & Lopes-Avila 1992, Mapes et al. 1992, Vanderlaan et al. 1990, Albro et al. 1979, Bushway et al. 1988, Fleeker & Cook 1991, Goh et al. 1991, Jung et al. 1989, Hammock & Mumma 1980) can eliminate several of these problems by providing a simple, on-site test for the mapping, remediation, and monitoring of affected areas.

We have developed a chromogenic immunoassay for PCB that is rapid, simple, sensitive, and cost effective. The test can be performed in the field, requires no special training, and can be completed in less than 20 minutes. Concentrations of PCB at 5 ppm and higher are detected. The real time testing of samples makes possible the more cost effective use of people, time, and equipment.

MATERIALS AND METHODS

Polyclonal antibodies - Immunogens were prepared by using 16 mg of 4-carboxylate derivative of pentachlorobiphenyl, activated by a modification of the mixed anhydride procedure of Vaughan & Osato 1952. Dimethylformamide was used as the solvent in a 2-5° C ice/water bath and the mixed anhydride was given ten minutes to form. This mixed anhydride solution was added to a solution of 70 mg of bovine albumin (BSA) in 5 mL of 200 mM sodium carbonate, pH 8.8. After two hours stirring at room temperature, the product was purified by dialysis versus phosphate buffered saline (pH 7.3). New Zealand white rabbits were immunized by intradermal and subcutaneous injections of 500 ug of this BSA conjugate in complete Freund's adjuvant. Subcutaneous booster injections of 250 ug of immunogen in incomplete Freund's adjuvant were given at two weeks and then on a monthly basis until a maximum response was obtained (approximately 3 months after initial injection). Polyclonal antisera were collected on a routine basis 7 to 14 days after each boost.

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Horseradish peroxidase (HRP) conjugate was prepared by reacting the 4-diazonium salt of pentachlorobiphenyl with HRP at pH 7 by the method of Kagan & Vallee 1969. The conjugate was purified by size exclusion chromatography and the hapten loading was determined spectrophotometrically to be between 2 and 4 moles per mole of HRP. A ratio between 0.65 and 0.82 in the absorbance at 330 and 404 nm indicates a loading of between 2 and 4 derivatives per HRP.

Antibody coated tubes were prepared by passively coating polystyrene (12X75 mm) tubes overnight at room temperature with rabbit anti-PCB antibody diluted in phosphate buffered saline (PBS, pH 7.3).

The assay procedure involves three steps: sample processing, immunological procedures, and assay interpretation. Sample processing involved shaking 10 g of soil with 20 mL of methanol. This step, the immunological procedures, and assay interpretation steps were identical to the PCP test (Mapes et al. 1992) with the following exceptions. The PCB-HRP conjugate was lyophilized, sealed in a glass ampule, and stored in a plastic dropper tube containing buffer to rehydrate the conjugate (recovery of the conjugate activity was between 80-100%). Just before the conjugate is added to the dilution tube, the glass ampule containing the HRP conjugate is broken and mixed with the buffer in the plastic dropper tube. The competitive immunoassay is initiated adding three drops (170 uL) of conjugate to each of the buffer tubes containing sample or standard, and pouring the contents into the antibody coated tubes. The rest of the assay protocol and interpretation of the results are unchanged.

RESULTS AND DISCUSSION

We have developed a fast, on-site immunoassay test for the semi-quantitation of PCB in soil. The format of the PCB soil test is a competitive ELISA (enzyme linked immunosorbent assay). Using this coated tube format, the amount of PCB in a sample is determined by comparison to the standard (Aroclor 1254), which is assayed in parallel with the sample. The standard is run in parallel to eliminate temperature and operator variations. This test is field compatible, semi-quantitative, specific, stable, reproducible, and detects PCB at concentrations as low as 5 ppm in soil. These claims are based on the following validation results.

1. The test is sensitive to 5 ppm for Aroclors 1260, 1254, 1248, 1242, 1232; 10 ppm for Aroclor 1016; and 20 ppm for Aroclor 1221.
2. The assay is highly specific for PCB.
   - "PCB-free" soils show little interference which indicates little matrix effect
   - Few compounds cross-react in the assay.
3. The assay is reproducible from person-to-person, lot-to-lot, and day-to-day.
4. The test can be stored at room temperature (20-30° C).
5. The assay can be performed at temperatures between 15-30° C.
6. Good correlation of the immunoassay to the reference GC methods was demonstrated with field samples.

The development of a soil test that can detect 5 ppm PCB is sufficiently

sensitive to meet most of the limits imposed by regulatory agencies. To develop the test at this level, the concentration of the standard was set such that samples containing 5 ppm or more PCB were positive. In addition, the dilution sequence was adjusted so that 5 ppm PCB was in the middle of the dose response curve. This was desirable since this area of the curve is the most precise. Also, the dilution was necessary to reduce the methanol extraction reagent to 5% in an aqueous buffer which dilutes the sample by 40 fold (2 fold for dilution into methanol and 20 fold for dilution into buffer). Therefore the dose being detected with the assay, when based on the concentration in the antibody coated tube, is less than 100 ppb (Aroclor 1254). Figure 1 shows the response of the assay when PCB (Aroclor 1254) was spiked into negative sample extracts. The figure shows that the test detects samples that contain 5 ppm or more Aroclor 1254. The assay detects the most common Aroclors; 1260, 1254, 1248, 1242, and 1232; at 5 ppm and the less common Aroclors 1221 and 1016 at 10 ppm and 20 ppm, respectively. Since our test is the most sensitive toward the more abundant Aroclors, it is expected that the test will detect samples containing 5 ppm or more PCB.

In addition to sensitivity, specificity is the other factor that greatly influences the performance of an immunoassay. Determination of the PCB assay specificity involved measuring the effect of soil matrices on the assay and evaluating the cross-reactivity of a number of compounds that might be expected to be found in conjunction with PCB contamination or compounds that are chemically similar to PCB. Matrix interference was investigated by determining the effect of "PCB-free" soils on the zero dose signal (Bo) and the effect of "PCB-free" soil extracts on the recovery of PCB. Nine soil matrices of different soil types, obtained from different parts of the U.S., were extracted and assayed. The range of Bo signal compared to extraction solvent was 101 % ("Ohio", a sandy loam type obtained from the Cincinnati area) to 90 % (Silty Loam). By this criterion the soil matrices have little effect on the immunoassay. To determine whether the soil extracts altered the assay sensitivity, the Ohio and Silty Loam soil extracts were spiked with PCB. Figure 2 shows the sensitivity profiles for the two soils compared to the extraction solvent. From these data we conclude that these two soils, the extremes in their effect on Bo signal, do not alter the sensitivity of the immunoassay. The results of both sets of experiments demonstrate that the assay is not significantly affected by soil matrices.

The results of the cross-reactivity studies showed that the assay is highly specific. Transformer fluids (Diala AX, R-Temp, E-Temp, mineral oil) do not interfere with the assay. Trichloroethylene, benzene, toluene, and most pesticides did not cross-react to any extent. However, some chlorinated aromatic compounds and some chlorinated aromatic pesticides do cross-react with the test. Even for the most cross-reactive compound the concentration that would give a positive assay when testing for 50 ppm PCB is 250 ppm. It is unlikely that concentrations at these levels and higher would be found at PCB contaminated sites.

Comparing the sensitivity profile curves generated by two people have shown that the reproducibility of the assay from person-to-person is acceptable. Studies comparing reproducibility of different lots of reagents are shown in Figure 1. These comparisons demonstrate little variation exist between lots that were manufactured according to standard operating procedures. The

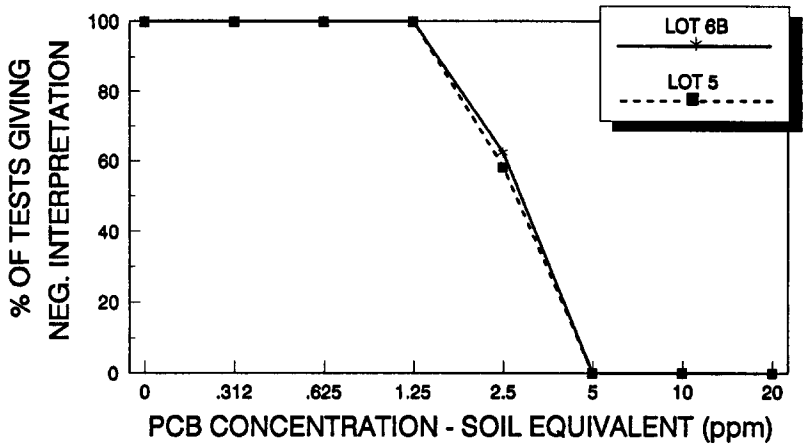

Figure 1. PCB RISc™ soil sensitivity profile. The immunoassay was performed according to the protocol. "PCB-free" soil extracts (10 g soil extracted with 20 mL methanol) were spiked with Aroclor 1254 at the concentrations indicated, treated as samples, and assayed. The results are presented as the percentage of assays that were interpreted as negative. Each point is the average of 2 different soil extracts repeated 8 times giving a total of 16 assays/point.

assays for the comparison of person and lot variation were performed on different days. Therefore, day-to-day variation was also negligible.

For field compatibility, an assay must be able to be performed at a variety of temperatures. The PCB soil test can be used at temperatures between 13-30° C with no significant change in assay performance. At 4° C the assay is more sensitive and the incidence of false positives increases when testing "PCB-free" soil samples. At 37° C the assay sensitivity is unaltered but performance is less precise. Consequently, the test should be performed at temperatures between 15 and 30° C.

Storage stability studies of the test kits presented in Figure 3 show that the kit is stable for greater than 3 months when stored at room temperature (20-25° C). The extrapolated values from these curves would suggest that the product will be stable for an extended period of time.

The initial emphasis of our work was to establish the performance of the assay with buffer and negative soil samples. However, the most important aspect of the assay development process is to verify the results by testing field samples and by testing the assay at field sites. A field study was performed by R. F. Weston, an environmental consulting company. They tested 30 soil samples with PCB concentrations ranging from non-detectable to 710 ppm. Most of the samples contained less than 100 ppm. Their results showed that the test had an overall accuracy of greater than 85 % compared to their GC results (SW 846, 3540/8080 with ECD detection). Internal testing of additional field samples have confirmed the results of the Weston study. The overall results of both studies showed only 11 % false positive and 4 % false negative results at the 50 ppm level with over 50 different samples tested. When testing at the 5 ppm level, the results were 91 % correct overall (7 %

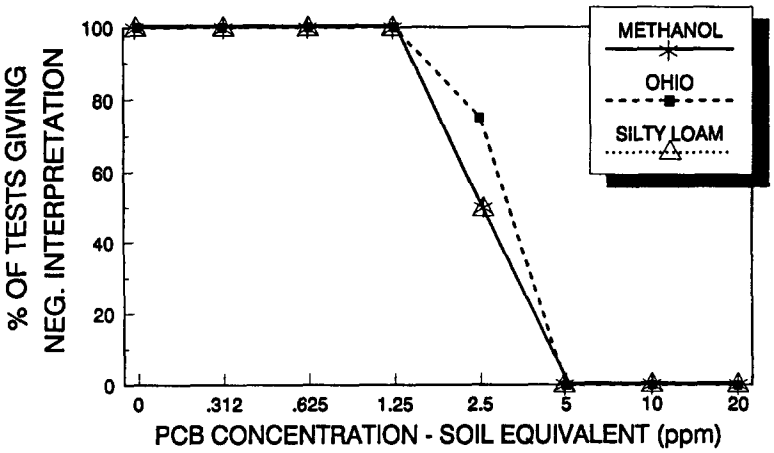

Figure 2. Comparison of the effects of different soil matrices on the sensitivity of the immunoassay. The soil extracts and the methanol extraction solvent control were spiked with the indicated concentrations of Aroclor 1254. The spiked materials were treated as samples and assayed. Each point was replicated 4 times. "Ohio" soil was obtained from the Cincinnati area and is a sandy loam type. Of the nine soils tested, these two soils were the most different in their effects on Bo and were therefore chosen for PCB sensitivity testing.

false positives, 2 % false negative). These correlation results justify the use of this kit as a field screening method for PCB contamination in soil samples.

Our primary objective was to develop an assay with the ability to quickly and easily screen for PCB in the field while detecting all positive samples with greater than 5 ppm PCB. This objective necessitated developing a test that was specific to PCB, rather than to Aroclor or congener, semi-quantitative rather than quantitative, and biased so that few if any positive samples would remain undetected.

PCB is made up of hundreds of different chlorinated biphenyl compounds. The diversity of the compounds presents a unique challenge to the development of an immunoassay that detects all of the compounds of interest with equal sensitivity. While our immunoassay does not quite accomplish this criterion, it does detect with the highest sensitivity the most common Aroclors. The better sensitivity to the more highly chlorinated Aroclors is not surprising since the antibodies were raised against pentachloro-congener found in the highest concentrations in Aroclors 1242-1260. Even though the test is more sensitive toward highly chlorinated Aroclors, there is only a four fold difference in the sensitivity toward any of the Aroclors on the list of Priority Group One Chemicals of the ATSDR that lists hazardous substances found at NPL sites (Fed. Reg. 1987). This immunoassay test kit will detect the PCB in any of these Aroclors if their concentration exceeds 20 ppm.

Making the assay semi-quantitative allowed the test to be easy to perform and to be field compatible. This was accomplished by using one standard against which the samples were compared. Most quantitative immunoassays use several standards and require extensive data manipulation and expensive

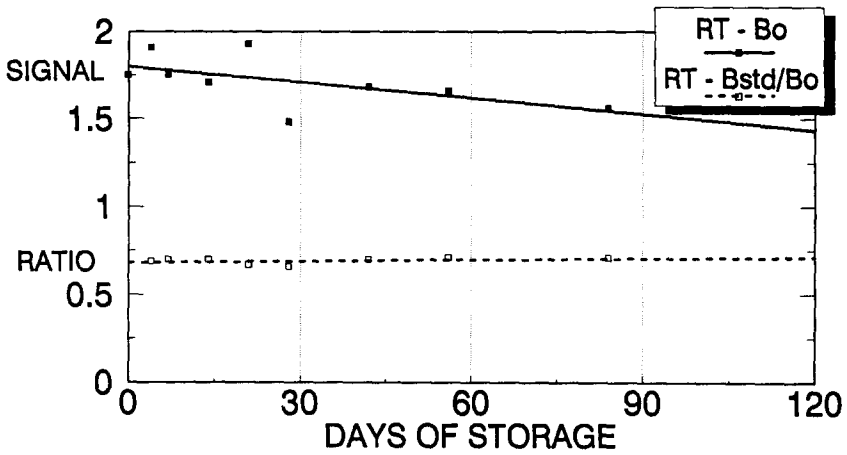

Figure 3. The stability of the test kit was determined at the time points indicated when the test was stored at room temperature (20-25° C). The stability is measured by determining the signal at zero dose PCB (Bo) and the signal of the kit standard (Bstd). The ratio of Bstd/Bo monitors changes in the immunochemistry.

instrumentation. The use of one standard eliminates the need for data manipulation, expensive instrumentation, but also limits the operator to determining the concentration as a range rather than a single value, e.g., <5 ppm, 5 to 50 ppm, or >50 ppm. In a field screening test, ranging the concentration of PCB in a sample should be sufficient for most decisions regarding disposition of the sample.

The test was biased to minimize false negative results. False negative results are potentially dangerous to the environment since they allow contamination to go undetected. A false positive on the other hand may be inconvenient but not dangerous. Therefore the test has been designed to detect samples having 5 ppm or greater PCB with a minimum number of false positive samples.

While these objectives impose certain limitations on the test, they make possible the rapid screening of samples in the field at relevant regulatory levels. The screening of multiple samples is a significant advantage compared to the standard analytical GC or GC-MS methods. The almost instant screening of a sample allows important questions to be answered concerning the project, e.g. has the plume of contamination been located; has the area been remediated to acceptable levels; does additional soil need to be removed; etc? Because this testing system rapidly answers these questions, personnel, time, and equipment can be used more cost effectively.

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